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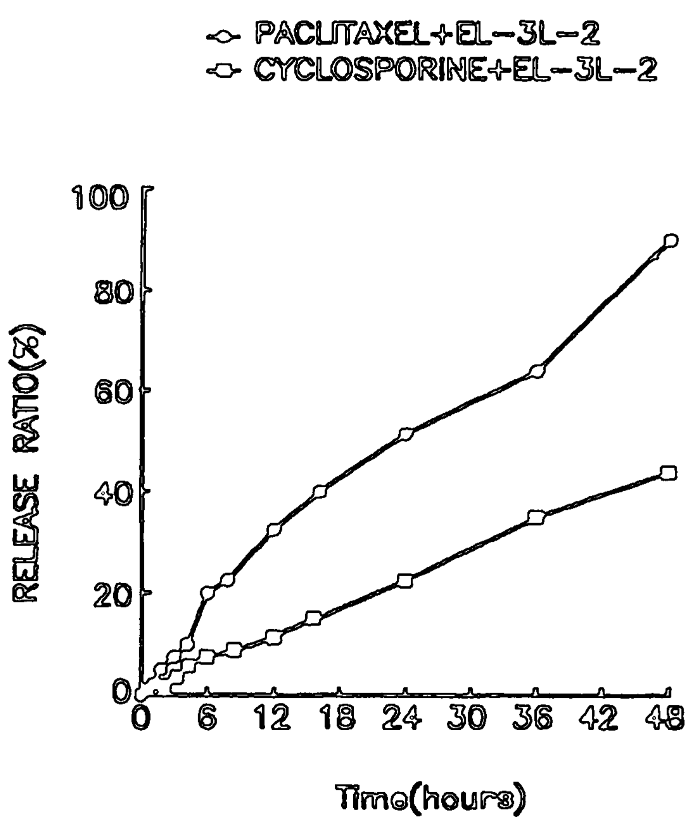
(54) Title: COPOLYMERIC MICELLE DRUG COMPOSITION AND METHOD FOR THE PREPARATION THEREOF

(57) Abstract

The present invention relates to a miscellar drug delivery system comprising a block copolymer having both hydrophobic and hydrophilic blocks; wherein the hydrophobic block is a biodegradable hydrophobic polymer selected from the group consiting of polylactide, polyglycolide, poly(lactide glycolide), polycaprolactone and derivatives thereof; and the hydrophilic polymer is poly(alkylene oxide). A hydrophobic drug can be readily incorporated into the micelle by using a simple method to obtain a therapeutically effective drug composition.



that constitutes the core, so as to mimic stabilized polymeric micelles. However, a crosslinking agent, or other means such as UV and heating with or without added peroxides, must be used in order to introduce crosslinking to the hydrophobic component of the block copolymer. Moreover, the biocompatibility or the safety of such crosslinked polymer particles has not yet been established.

There have been reported other studies on biodegradable block copolymer micelles having surfactant-like properties, and particularly noteworthy are the attempts to incorporate hydrophobic drugs into block copolymer micelles stabilized due to the specific nature and properties of the copolymer.

For example, EP No. 0 397 307 A2 discloses micelles of an AB type diblock copolymer which contains poly(ethylene oxide) as the hydrophilic component and poly(amino acid), e.g., polyaspartic acid, polyglutamic acid and polylysine, as the hydrophobic component, wherein therapeutically active agents are chemically bonded to the hydrophobic component of the polymer. However, it is difficult to prepare a polymer bearing specified functional groups, and there also exists the problem that such composition having a chemically bonded drug may not be safe for human use.

EP No. 0 583 955 A2 discloses a method for physically incorporating hydrophobic drugs into diblock copolymer micelles described in EP No. 0 397 307 A2. This method, thus, solves the potential safety problem arising from chemically bonding drugs to micelles. However, the poly(amino acid) segment may induce an immunoreaction and the use of an organic solvent in the preparation of the formulation may pose a problem. Further, because the peptide bonds are cleaved by enzymes in the body, it is difficult to control the release rate of the drug incorporated therein.

Accordingly, the present inventors have endeavored to develop an improved drug delivery system which is free of the problems mentioned above, and unexpectedly found that block copolymer micelles, composed of poly(thylene oxide) as the hydrophilic component and polylactide, polycaprolactone,

COPOLYMERIC MICELLE DRUG COMPOSITION AND METHOD FOR THE PREPARATION THEREOF

Field of the Invention

The present invention relates to a drug delivery system, and more particularly, to a polymeric micelle drug composition comprising a hydrophobic drug and a block copolymer having a hydrophilic polymer component and a hydrophobic biodegradable polymer component.

Background of the Invention

Many important drugs are hydrophobic and have limited solubilities in water. In order to attain the expected therapeutic effect of such drug, it is usually required that a solubilized form of the drug is administered to a patient. For this purpose, there have been developed a number of methods, which are based on the use of: auxiliary solvents; surfactants; soluble forms of the drug, e.g., salts and solvates; chemically modified forms of the drug, e.g., prodrugs; soluble polymer-drug complexes; special drug carriers such as liposome; and others. However, because each of the above methods is hampered by one or more particular problems, e.g., the method based on the use of surfactant micelles to solubilize hydrophobic drugs has problems in that most of the surfactants are relatively toxic and that precipitation of the surfactant occurs when subjected to dilution.

To solve above-mentioned problems associated with surfactants, EP No. 0 552 802 A2 discloses a method for preparing micelle-shaped polymer particles by chemically fixing micelles having poly(ethylene oxide) as the hydrophilic component and a biodegradable polymer block which can be crosslinked in an aqueous phas as the hydrophobic component. That is, chemically fixed polymer particles ar prepared by chemically crosslinking the hydroph bic component

Fig. 3 shows the release profiles of paclitaxel and cyclosporin incorporated in the EL-3L-2 copolym r micelle.

Fig. 4 shows the anticancer activity of paclitaxel incorporated in the EL-2L-2 copolymer micelle.

Detailed Description of the Invention

The drug delivery system of the present invention comprises block copolymer micelles made of biodegradable polymers, and when administered, it decomposes *in vivo* into non-toxic small molecules by simple hydrolysis or by the action of enzymes. Biodegradable block copolymer micelles having an average diameter of 10 to 40 nm, are particularly suitable for formulating an injection composition of hydrophobic drugs which are either insoluble or only slightly soluble in water.

The block copolymer micelle of the present invention may be prepared by combining a biodegradable hydrophobic polymer e.g., polylactide(PLA), polycaprolactone(PCL), poly(lactide glycolide)(PLGA), polyglycolide (PGA) and derivatives thereof with a hydrophilic polymer such as poly(alkylene oxide). A hydrophobic drug may be delivered to a patient much more effectively when it is carried by the block copolymer micelle of the present invention and the sustained release of the drug stored in the micelle enhances the therapeutic effect of the drug.

The block copolymer used in the drug composition of the present invention may be a polymer of formula (I) or (II):

$$R_1\text{-}(\text{-}OCH_2CH_2\text{-})_n\text{-}X \qquad (I)$$

$$X\text{-}(\text{-}OCH_2CH_2\text{-})_n\text{-}X \qquad (II)$$

wherein,

R_1 is hydrogen or C_{1-20} alkyl, preferably it is C_{1-5} alkyl;

m is an int ger larger than 2, preferably from 10 to 3,000; and

poly(lactide-glycolide), polyglycolid or a mixture thereof as the hydrophobic component, is very effective in solubilizing hydrophobic drugs by physically incorporating them therewithin. The resulting micelle-drug composition is suitable for sustained-release of the drug in vivo, thereby enhancing the therapeutic effect of the drug. Such effect may be maximized by controlling the molecular weights and the relative ratio of the hydrophilic and hydrophobic blocks.

Summary of the Invention

Accordingly, it is an object of the present invention to provide an effective carrier of hydrophobic drugs which may be used in preparing a pharmaceutically effective drug composition.

In accordance with one aspect of the present invention, there is provided a polymeric micelle drug composition which comprises a polymeric micelle drug composition comprising: a micelle of a block copolymer having a hydrophilic component and a hydrophobic component; and at least one hydrophobic drug incorporated into the micelle; wherein the hydrophobic component is a biodegradable polymer selected from the group consisting of polylactide, polyglycolide, poly(lactide glycolide), polycaprolactone, and a mixture thereof; and the hydrophilic component is poly(alkylene oxide).

Brief Description of Drawings

Fig. 1 is the GPC(gel permeation chromatography) trace of the polylactide-poly(ethylene oxide)-polylactide triblock copolymer(EL-3L-1)(column:MT3-MT4(Waters, U.S.A.), flow rate :10 ml/min, eluent: tetrahydrofuran).

Fig. 2 is the GPC(gel permeation chromatography) trace of the poly(ethylene oxide)-polycaprolactone diblock copolymer (EC-2C-1)(column:Asahipak GS 520H, lu nt: distilled wat r).

$$-[-OCO(CH_2)_5-]_n \qquad \text{(VIII)}$$

wherein,

R_2 and R_3 are independently H or CH_3;

x and y are independently integers larger than 2; and

n is an integer larger than 2, preferably from 2 to 500.

Diblock and triblock copolymers(AB type and ABA type) may be composed of a poly(ethylene oxide)(PEO) hydrophilic component(B) of and a polylactide(PLA) hydrophobic component(A), as shown in formulae (IX) and (X):

$$R_1-(OCH_2CH_2-)_m-[-OCO\underset{CH_3}{CH}-)_i-OH \qquad \text{(IX)}$$

$$H-[O\underset{CH_3}{CH}CO-]_j-(-OCH_2CH_2-)_m-[-OCO\underset{CH_3}{CH}-]_k-OH \qquad \text{(X)}$$

wherein,

i, j, k and m are as described above.

Diblock or triblock copolymer of the present invention may be prepared by ring-opening polymerization. For example, the AB type diblock copolymer composed of PEO as the hydrophilic component(B) and PLA as the hydrophobic component(A) may be prepared by using PEO having a methoxy group at one terminal and a hydroxy group at the other terminal. The ABA type triblock copolymer may be prepared by using PEO having hydroxy groups at both terminals. The solubility of the micelle in water may be regulated by controlling the ratio of the hydrophilic component and hydrophobic component.

Suitable hydrophobic drugs which may be incorporated into the block copolymer micelle of the present invention are anti-cancer drugs such as paclitaxel, doxorubicin, teniposide, etoposide, daunomycin, methotrexate, mitomycin C and the like; antiphlogistic anodynes such as indomethacin, ibuprofen and the like; immunosuppressants such as cyclosporin and the like; hepatism remedies such as biphenyldimethylcarboxylate and the like; hormone compositions; antibiotics; chemoth rapeutics; metabolic pharmaceuticals; digestive disease remedies; respiratory

X is a biod gradable hydrophobic polymer segment having a mol cular w ight more than 100, pr ferably 300-100,000, and it is preferably sel cted from the group consisting of polylactide(PLA), polycaprolactone(PCL), poly(lactide glycolide)(PLGA), polyglycolide(PGA) and derivatives thereof.

The more preferable block copolymer which may be used in the drug composition of the present invention are di- or tri-block copolymers of formulae (III), (IV), (V) and (VI):

$$R_1\text{-}(\text{-}OCH_2CH_2\text{-})_m\text{-}[\text{-}OCO\underset{CH_3}{CH}\text{-}]_i\text{-}OH \qquad \text{(III)}$$

$$H\text{-}[\text{-}OCO\underset{CH_3}{CH}\text{-}]_j\text{-}(\text{-}OCH_2CH_2\text{-})_m\text{-}[\text{-}OCO\underset{CH_3}{CH}\text{-}]_k\text{-}OH \qquad \text{(IV)}$$

$$R_1\text{-}(\text{-}OCH_2CH_2\text{-})_m\text{-}[\text{-}OCO(CH_2)_5\text{-}]_l\text{-}OH \qquad \text{(V)}$$

$$H\text{-}[\text{-}O(CH_2)_5CO\text{-}]_n\text{-}(\text{-}OCH_2CH_2\text{-})_m\text{-}[\text{-}OCO(CH_2)_5\text{-}]_p\text{-}OH \qquad \text{(VI)}$$

wherein,

R_1 is hydrogen or C_{1-20} alkyl, preferably it is C_{1-5} alkyl;

i is an integer larger than 2, preferably from 2 to 1,500;

j and k are independently integers larger than 1, preferably from 2 to 1,000;

l is an integer larger than 2, preferably from 2 to 700;

m is as described above; and

n and p are independently integers larger than 1, preferably from 2 to 500.

As described above, while poly(ethylene oxide) may be used as the preferred hydrophilic component of the block copolymer of the present invention, the hydrophobic component of the block copolymer of the present invention may comprise polylactide, polyglycolide, poly(lactide glycolide), polycaprolactone, derivatives thereof and the like having the following structures:

$$\text{-}[\text{-}OCO\underset{R_2}{CH}\text{-}]_x\text{-}[OCO\underset{R_3}{CH}\text{-}]_y\text{-}OH \qquad \text{(VII)}$$

A block copolymer is added to an organic solution of a drug and the mixture is dialyzed against a buffer solution and th n water.

In the solvent evaporation or the dialysis method, suitable organic solvents for dissolving drugs are dimethylformamide(DMF), dimethylsulfoxide(DMSO), dioxane, chloroform, n-hexane, toluene, dichloromethane, ethyl acetate and the like.

The block copolymers of the present invention form stable micelles having an average size of 10-60 nm as shown in Table 1 of the Examples. Micelles of this size range are suitable for injection formulations and can avoid RES uptake while exerting EPR effect. The stability of the micelles is excellent, as can be seen from the gel permeation chromatography shown in Figure 2.

Further, a hydrophobic drug may be incorporated into the block copolymer micelle of the present invention by methods other than those described above, wherein the amount and physical state of the incorporated drug may vary depending on the composition of the block copolymer and also on the method of preparing the polymer micelle(Table 1). As the drug held in the compact core of the hydrophobic component is released <u>in vivo</u> in a controlled manner, the composition of the present invention is partially suitable for formulating drugs which are not amenable to conventional formulating techniques.

For example, paclitaxel is an outstanding anti-cancer agent but formulation thereof is difficult, mainly due to its low water-solubility. For this reason, a paclitaxel formulation containing Cremophor EL as the adjuvant is currently on the market, although Cremophor EL may cause some serious side effects. This particular formulation has other problems: i.e., it tends to form minute precipitates which require the us of a filter in the injection lin ; and the requir d period of administration is long, about 24 hours.

disease rem di s; anti-allergic pharmaceuticals; central nervous system dis ase remedies; peripheral disease remedies; circulatory disease remedies; but not limited to those mentioned above.

In order to incorporate one or more drugs mentioned above into the block copolymer micelle, various methods described below may be used alone or in combination.

(1) Stirring

A drug is added to an aqueous solution of a block copolymer, and stirred for 2 to 24 hours to obtain micelles containing the drug.

(2) Heating

A drug and an aqueous solution of a block copolymer are mixed and stirred at 40 to 120 °C for 5 minutes to 24 hours and then cooled to room temperature while stirring to obtain micelles containing the drug.

(3) Ultrasonic Treatment

A mixture of a drug and an aqueous solution of a block copolymer is subjected to an ultrasonic treatment for 1 second to 1 hour and then stirred at room temperature to obtain micelles containing the drug.

(4) Solvent Evaporation

A drug is dissolved in an organic solvent and added to an aqueous solution of a block copolymer. Subsequently, the organic solvent was evaporated slowly while stirring, and then, filtered to remove non-solubilized drug.

(5) Dialysis

Preparation Example 1: Synthesis of Polylactide-Poly(ethylene oxide)-Polylactide Triblock Copolymer (EL-3L-0)

2 g of poly(ethylene glycol)(Mw 3400) was dried under a reduced pressure at 120 °C for 2 hours and 0.59 mg of stannous octoate(amount corresponding to 0.1% of D,L-lactide) was added thereto as a catalyst. The resulting mixture was subjected to a reduced pressure at 100 °C for 20 to 30 minutes to remove volatile compounds, mixed with 0.5882 g of D,L-lactide, and the mixture was reacted at 130 °C for 13 hours.

The block copolymer thus obtained was dissolved in 10 ml of chloroform and then an excess amount of diethyl ether was added with stirring to induce precipitation of the polymer. The precipitate was filtered and washed several times with diethyl ether, and then dried under a reduced pressure at 30 °C for one day to obtain 2.46 g of a triblock copolymer, polylactide-poly(ethylene oxide)-polylactide(PLA-PEO-PLA), designated EL-3L-0(yield 93%). The properties of this block copolymer are listed in Table 1 and the results of gel permeation chromatography are shown in Fig. 1.

Preparation Example 2: Synthesis of Polylactide-Poly(ethylene oxide)-Polylactide Triblock Copolymer (EL-3L-1)

The procedure of Preparation Example 1 was repeated, except for using 2 g of poly(ethylene glycol)(Mw 3400) and 1.18 g of D,L-lactide, to obtain 2.95 g of a triblock copolymer, polylactide-poly(ethylene oxide)-polylactdie(PLA-PEO-PLA), designated EL-3L-1(yield 93%). The properties of this block copolymer are listed in Table 1.

In contrast,the block copolymer micelle of th present invention greatly enhances the solubility of paclitaxel, and th micelle-paclitaxel composition thus obtained is essentially non-toxic and exhibits enhanced anti-cancer therapeutic activity. As shown in Table 2, the amount of paclitaxel incorporated into the particular block copolymer micelles was 25.16 ± 3.27 %, while that of cyclosporin was 23.13 ± 2.31 %(Table 3). Further, the micelle-paclitaxel composition of the present invention released 85 % of the incorporated paclitaxel continuously over a period of 48 hours, while effectively preventing the cancer cells from growing. In case of the micelle-cyclosporin immunosuppressant composition, 40 % of the active ingredient was released continuously over a period of 48 hours.

The biodegradable diblock or triblock copolymer of the present invention can form stable micelles which can incorporate hydrophobic drugs therewithin. The present invention thus provides a micelle-drug composition which is therapeutically more effective, and toxicologically much safer, than conventional formulations of hydrophobic drugs.

The following Preparation Examples and Examples are provided for purposes of illustrating certain aspects of the present invention only; they are not to be construed as limiting the scope of the present invention in any way.

Preparation Example 6: Synthesis f Poly(ethylene oxide)-P lylactide Diblock Copolymer (EL-2L-1)

The procedure of Preparation Example 1 was repeated, except for using 2 g of monomethoxy poly(ethylene glycol)(Mw 2000) and 1.0 g of D,L-lactide, to obtain 2.70 g of a diblock copolymer of poly(ethylene oxide)-polylactdie(PEO-PLA), designated EL-2L-1(yield 90%). The properties of this block copolymer are listed in Table 1.

Preparation Example 7: Synthesis of Poly(ethylene oxide)-Polylactide Diblock Copolymer (EL-2L-2)

The procedure of Preparation Example 1 was repeated, except for using 2 g of monomethoxy poly(ethylene glycol)(Mw 2000) and 1.5 g of D,L-lactide, to obtain 3.22 g of a diblock copolymer of poly(ethylene oxide)-polylactdie(PEO-PLA), designated EL-2L-1(yield 92%). The properties of this block copolymer are listed in Table 1.

Preparation Example 8: Synthesis of Polycaprolactone-Poly(ethylene oxide)-Polycaprolactone Triblock Copolymer (EC-3C-1)

The procedure of Preparation Example 1 was repeated, except for using 2 g of poly(ethylene glycol)(Mw 3400) and 1.1765 g of caprolactone, to obtain 2.86 g of a triblock copolymer of polycaprolactone-poly(ethylene oxide)-polycaprolactone(PCL-PEO-PCL), designated EC-3C-1(yield 90%). The properties of this block copolymer are listed in Table 1.

Preparation Example 3: **Synthesis of Polylactide-P ly(ethylen oxide)-Polylactide Triblock Copolymer (EL-3L-2)**

The procedure of Preparation Example 1 was repeated, except for using 2 g of poly(ethylene glycol)(Mw 3400) and 1.76 g of D,L-lactide, to obtain 3.46 g of a triblock copolymer, polylactide-poly(ethylene oxide)-polylactdie(PLA-PEO-PLA), designated EL-3L-2(yield 93%). The properties of this block copolymer are listed in Table 1.

Preparation Example 4: **Synthesis of Polylactide-Poly(ethylene oxide)-Polylactide Triblock Copolymer (EL-3L-3)**

The procedure of Preparation Example 1 was repeated, except for using 2 g of poly(ethylene glycol)(Mw 3400) and 2.35 g of D,L-lactide, to obtain 3.87 g of a triblock copolymer of polylactide-poly(ethylene oxide)-polylactdie (PLA-PEO-PLA), designated EL-3L-3(yield 89%). The properties of this block copolymer are listed in Table 1.

Preparation Example 5: **Synthesis of Poly(ethylene oxide)-Polylactide Diblock Copolymer (EL-2L-0)**

The procedure of Preparation Example 1 was repeated, except for using 2 g of monomethoxy poly(ethylene glycol)(Mw 2000) and 0.5 g of D,L-lactide, to obtain 2.28 g of a diblock copolymer of poly(ethylene oxide)-polylactdie(PEO-PLA), designated EL-2L-0(yield 91%). The properties of this block copolymer are listed in Table 1.

Table 1

Copolymer	Calculated Composition	Measured Composition[a]	Yield (%)	Solubility (g/100ml)	Size[b] (nm)
EL-3L-0	PLA500-PEO3400-PLA500	PLA467-PEO3684-PLA467	95	over 20	13.4 ± 3.6
EL-3L-1	PLA1000-PEO 3400-PLA1000	PLA856-PEO3684-PLA856	93	over 20	21.1 ± 2.8
EL-3L-2	PLA1500-PEO 3400-PLA1500	PLA1402-PEO3684-PLA1402	92	3.5	41.2 ± 3.1
EL-3L-3	PLA2000-PEO 3400-PLA2000	PLA1876-PEO3684-PLA1876	89	0.2	38.3 ± 2.8
EL-2L-0	mPEO2000-PLA500	mPEO2141-PLA457	91	over 20	12.4 ± 1.2
EL-2L-1	mPEO2000-PLA1000	mPEO2141-PLA916	90	over 20	25.3 ± 2.4
EL-2L-2	mPEO2000-PLA1500	mPEO2141-PLA1367	92	over 20	35.4 ± 3.1
EC-3C-1	PCL1000-PEO 3400-PCL1000	PCL921-PEO3684-PCL921	90	9.6	45.1 ± 3.6
EC-2C-1	mPEO2000-PCL1500	mPEO2141-PCL1387	91	4.0	52.5 ± 2.9

a: ^{1}H NMR(solvent:$CDCl_3$)

b: dynamic light scattering

Preparation Example 9: Synthesis f Poly(ethylene oxide)-Polycaprolactone Diblock Cop lymer (EC-2C-1)

The procedure of Preparation Example 1 was repeated, except for using 2 g of monomethoxy poly(ethylene glycol)(Mw 2000) and 1.5 g of caprolactone, to obtain 3.2 g of a diblock copolymer of poly(ethylene oxide)-polycaprolactone(PEO-PCL), designated EC-2C-1(yield 91%). The properties of this obtained block copolymer are listed in Table 1.

Preparation Example 10: Preparation of Polymeric Micelle

Each of the block copolymers synthesized in Preparation Example 1-9 was dissolved in distilled water or 0.1 M phosphate buffer(pH 7.4) to a concentration of 0.01 to 5 %(w/v) to obtain a polymeric micelle solution. The size of the micelle in each polymeric micelle solution measured by dynamic light scattering method was in the range from 10 to 60 nm as shown in Table 1. Polymeric micelle of this size is suitable for use as a drug carrier. The formation of the polymeric micelle was confirmed by the gel permeation chromatography in Fig. 2.

(3) Incorporation by Dialysis

5 mg of paclitaxel was dissolved in 5 ml of DMF. EL-3L-2 synthesized in Pr paration Example 3 was added to the resulting solution and the mixture was stirred overnight. The mixture was dialyzed against 0.1 M phosphate buffer(pH 7.4) for 5 hours using a dialysis membrane(MWCO: 12000), and then against distilled water for 5 hours. The dialyz d solution was filtered with a 0.45 μm membrane filter and a clear solution of block copolymer micelles containing paclitaxel was obtained. This procedure was repeated using EL-2L-2 and EC-3C-1 synthesized in Preparation Example 7 and 8. The results are shown in Table 2.

These experiments shows that paclitaxel can be readily incorporated into the inventive polymeric micelles in an amount of upto 25.16 ± 3.23 %.

Table 2

Copolymer	Paclitaxel Incorporation Ratio(%)		
	Stirring	Solvent Evaporation	Dialysis
EL-3L-2	4.58 ± 0.36	15.53 ± 1.97	10.89 ± 1.57
EL-2L-2	5.25 ± 0.46	18.44 ± 2.18	14.14 ± 1.94
EC-3C-1	2.13 ± 0.22	25.16 ± 3.23	13.05 ± 1.63

Example 2 : Preparation of Block Copolymer Micelle Containing Cyclosporin

(1) Incorporation by Solvent Evaporation

10 mg of cyclosporin A, an immunosuppressant which is hardly-soluble in water, was dissolved in 1 ml of N,N-dimethyl acetamide and added slowly to a solution containing 20 mg of EL-3L-2 in 20 ml of distilled water. The resulting

Example 1: Preparation of Block Cop lymer Micelle C ntaining Paclitaxel

(1) Incorporation of paclitaxel into EL-3L-2, EL-2L-2 and EC-3C-1 by the stirring method

10 mg of each of the block copolymer EL-3L-2, EL-2L-2 and EC-3C-1 synthesized in Preparation Example 3, 7 and 8 was dissolved in 3 ml of distilled water and 5 mg of paclitaxel, an anticancer drug which is hardly-soluble in water, was added thereto and stirred for 2 hours. The resulting solution was filtered with a 0.45 μm membrane filter to remove unsolubilized paclitaxel and a clear solution of block copolymer micelles containing paclitaxel was obtained. The amount of paclitaxel incorporated into the polymeric micell was determined by HPLC(column:Curosil-PFP(4.6*250 mm, 5 μm particle size, Phenomenex, U.S.A.), mobile phas : acetonitrile/distilled water=45:55%(v/v)). The results are shown in Table 2.

(2) Incorporation by Solvent Evaporation

EL-3L-2 synthesized in Preparation Example 3 was dissolved in distilled water, and a chloroform solution containing 3 mg of paclitaxel is slowly added thereto. The resulting mixture was stirred at room temperature overnight while allowing chloroform to evaporate. The resulting solution was filtered with a 0.45 μm membrane filter to remove unsolubilized paclitaxel and a clear solution of block copolymer micelles containing paclitaxel was obtained. This procedure was repeated using EL-2L-2 and EC-3C-1 synthesized in Preparation Example 7 and 8. The results are shown in Table 2.

Example 3: Release Test

5 mℓ each of the paclitaxel- and the cyclosporin-containing EL-3L-2 copolymer micelle solution prepared in Examples 1 and 2, was placed in a dialysis sack(MWCO: 12,000). The sack was put into 1 ℓ of H_2O, and the amount of paclitaxel or cyclosporin released from the micelles was determined relative to the time. As can be seen from Fig. 3, the incorporated drugs show sustained release profiles.

Example 4: Toxicity and Efficacy Test

10^6 P388 leukemia cells were injected intraperitoneally to each member of three groups of mice, each consisting of six female BDF1 mice weighing 22 to 25 g.

24 Hours after the administration of leukemia cells, each of the mice in Group I was injected intraperitoneally with a vehicle(5% DMSO and 5% Cremophor saline solution) in an amount of 12.5 mg/kg, four times at a 24-hour interval, and each of the mice in Group II, was treated similarly with paclitaxel and the vehicle(5% DMSO and 5% Cremophor saline solution) under the same conditions.

On the other hand, each of the mice in Group III was administered intraperitoneally with 25 mg/kg of paclitaxel-containing EL-2L-2 copolymer micelle solution prepared in Example 1 (2), twice at 24 and 72 hours after the administration of the leukemia cells,.

The average survival time and the weight change in day 5 are listed in Table 4.

mixture was stirred overnight at room temperature while allowing N,N-dim thyl acetamide to evaporate off and the r sulting solution was filtered with a 0.45 μm membrane filter to obtain a cl ar solution of th block copolymer micelles containing cyclosporin. This procedure was repeated using EC-2C-1 synthesized in Preparation Example 9. The results are shown in Table 3.

(2) Incorporation by Dialysis

10 mg of cyclosporin A was dissolved in 5 ml of DMF. 20 mg of EL-3L-2 synthesized in Preparation Example 3 was add d to the resulting solution and the mixture was stirred overnight. The mixture was dialyzed against 0.1 M phosphate buffer(pH 7.4) for 5 hours using dialysis membrane(MWCO: 12000), and then against distilled water for 5 hours. The dialyzed solution was filtered with a 0.45 μm membrane filter and a clear solution of block copolymer micelles containing paclitaxel was obtained. This procedure was repeated using EC-2C-1 synthesized in Preparation Example 9. The results are shown in Table 3.

These experiments shows that cyclosporin can be readily incorporated in the inventive polymeric micelles in an amount of upto 23.13 ± 2.31 %.

Table 3

Copolymer	Cyclosporin Incorporation Ratio(%)	
	Solvent Evaporation	Dialysis
EL-3L-2	17.76 ± 1.97	14.96 ± 1.67
EC-2C-1	23.13 ± 2.31	17.03 ± 1.84

What is claimed is:

1. A polymeric micelle drug composition comprising: a micelle of a block copolymer having a hydrophilic component and a hydrophobic component; and at l ast one hydrophobic drug incorporated into the micelle; wherein the hydrophobic component is a biodegradable polymer selected from the group consisting of polylactide, polyglycolide, poly(lactide glycolide), polycaprolactone, and a mixture thereof; and the hydrophilic component is poly(alkylene oxide).

2. The polymeric micelle drug composition of claim 1, wherein the block copolymer is a polymer of formula (I) or (II):

$$R_1\text{-}(\text{-}OCH_2CH_2\text{-})_m\text{-}X \qquad (I)$$

$$X\text{-}(\text{-}OCH_2CH_2\text{-})_m\text{-}X \qquad (II)$$

wherein

R_1 is hydrogen or $C_{1\text{-}20}$ alkyl;

m is an integer ranging from 2 to 3,000; and

X is a polymeric segment having a molecular weight ranging from 100 to 100,000, which is selected from the group consisting of polylactide(PLA), polyglycolide(PGA), poly(lactide glycolide)(PLGA), polycaprolactone(PCL) and derivatives thereof.

3. The polymeric micelle drug composition of claim 1, wherein the block copolymer is a polymer of formula (III) or (IV):

$$R_1\text{-}(\text{-}OCH_2CH_2\text{-})_m\text{-}[\text{-}OCO\underset{CH_3}{\underset{|}{C}}H\text{-}]_j\text{-}OH \qquad (III)$$

$$H\text{-}[\text{-}OCO\underset{CH_3}{\underset{|}{C}}H\text{-}]_j\text{-}(\text{-}OCH_2CH_2\text{-})_m\text{-}[\text{-}OCO\underset{CH_3}{\underset{|}{C}}H\text{-}]_k\text{-}OH \qquad (IV)$$

Tabl 4

Group	Administered solution	Average survival time (hours)	Weight change(g) (at day 5)
I	Vehicle	248±54	-4.9
II	Paclitaxel+vehicle	407±81	-13.9
III	Paclitaxel+EL-2L-2	520±94	-7.5

The anticancer activity of the paclitaxel-containing EL-2L-2 copolymer micelle was determined by measuring the tumor weights of the Group III mice relative to those of Groups I and II at a predetermined time. The result in Fig. 4 shows that the growth of tumor was efficiently inhibited by the polymeric micelle drug composition of the present invention.

As shown above, water-insoluble, hydrophobic drugs may be readily loaded into the biodegradable block copolymer micelles of the present invention having a hydrophilic component and a hydrophobic component by way of either stirring, heating, ultrasonic treatment, solvent evaporation, dialysis and the like. The polymeric micelle drug composition thus obtained has a greatly improved pharmaceutical efficacy because an increased amount of the drug may be transferred effectively in patient's body.

7. The polymeric micelle drug composition of claim 6, wher in the hydrophobic drug is select d from the group consisting of: paclitaxel, doxorubicin, and cyclosporin.

8. The polymeric micelle drug composition of claim 1, wherein the hydrophobic drug is paclitaxel and the hydrophobic component is polylactide or polycaprolactone.

9. A process for incorporating a hydrophobic drug into a block copolymer micelle comprising the steps of:

preparing a micelle solution of a block copolymer having a hydrophobic component and a hydrophilic component, the hydrophobic component being a biodegradable hydrophobic polymer selected from the group consisting of polylactide, polyglycolide, poly(lactide glycolide), polycaprolactone and a mixture thereof and the hydrophilic component being poly(alkylene oxide); and

incorporating the hydrophobic drug into the block copolymer micelle by mixing the hydrophobic drug with the block copolymer solution.

10. The process of claim 9, further comprising the step of subjecting the mixture of the hydrophobic drug and the block copolymer solution to stirring, heating, ultrasonic treatment, solvent evaporation or dialysis.

wherein,

R_1 is hydrogen or C_{1-20} alkyl;

i, j and k are independently integers ranging from 2 to 1,000; and

m is an integer ranging from 2 to 3,000.

4. The polymeric micelle drug composition of claim 1, wherein the block copolymer is a polymer of formula (V) or (VI):

$$R_1\text{-}(\text{-}OCH_2CH_2\text{-})_m\text{-}[\text{-}OCO(CH_2)_5\text{-}]_l\text{-}OH \qquad (V)$$

$$H\text{-}[\text{-}O(CH_2)_5CO\text{-}]_n\text{-}(\text{-}OCH_2CH_2\text{-})_m\text{-}[\text{-}OCO(CH_2)_5\text{-}]_p\text{-}OH \qquad (VI)$$

wherein,

R_1 is hydrogen or C_{1-20} alkyl;

l is an integer ranging from 2 to 700;

m is an integer ranging from 10 to 3,000; and

n and p are independently integers ranging from 2 to 500.

5. The polymeric micelle drug composition of claim 1, wherein the hydrophobic drug is selected from the group consisting of: anti-cancer drugs, antiphlogistic anodynes, immunosuppressants, hepatism remedies, hormone compositions, chemotherapeutics; metabolic pharmaceuticals; digestive disease remedies; respiratory disease remedies; anti-allergic pharmaceuticals; central nervous system disease remedies; peripheral disease remedies; and circulatory disease remedies.

6. The polymeric micelle drug composition of claim 1, wherein the hydrophobic drug is selected from the group consisting of: paclitaxel, doxorubicin, teniposide, etoposide, daun mycin, methotrexate, mitomycin C, indomethacin, ibuprofen, cyclosporin, and biphenyldimethylcarboxylate.

3. Th polym ric micelle drug composition of claim 1, wher in th block copolym r is a polymer of formula (III) or (IV):

$$R_1\text{-}(\text{-}OCH_2CH_2\text{-})_m\text{-}[\text{-}OCO\underset{CH_3}{CH}\text{-}]_i\text{-}OH \qquad \text{(III)}$$

$$H\text{-}[\text{-}OCO\underset{CH_3}{CH}\text{-}]_j\text{-}(\text{-}OCH_2CH_2\text{-})_m\text{-}[\text{-}OCO\underset{CH_3}{CH}\text{-}]_k\text{-}OH \qquad \text{(IV)}$$

wherein,

R_1 is hydrogen or C_{1-20} alkyl;

i, j and k are independently integers ranging from 2 to 1,000; and

m is an integer ranging from 2 to 3,000.

4. The polymeric micelle drug composition of claim 1, wherein the block copolymer is a polymer of formula (V) or (VI):

$$R_1\text{-}(\text{-}OCH_2CH_2\text{-})_m\text{-}[\text{-}OCO(CH_2)_5\text{-}]_l\text{-}OH \qquad \text{(V)}$$

$$H\text{-}[\text{-}O(CH_2)_5CO\text{-}]_n\text{-}(\text{-}OCH_2CH_2\text{-})_m\text{-}[\text{-}OCO(CH_2)_5\text{-}]_p\text{-}OH \qquad \text{(VI)}$$

wherein,

R_1 is hydrogen or C_{1-20} alkyl;

l is an integer ranging from 2 to 700;

m is an integer ranging from 10 to 3,000; and

n and p are independently integers ranging from 2 to 500.

7. The polymeric micelle drug composition of claim 1, wherein the hydrophobic drug is paclitaxel, doxorubicin or cyclosporin.

AMENDED CLAIMS

[received by the International Bureau on 22 January 1997 (22.01.97); original claims 5,6 and 10 cancelled; original claims 1,7 and 9 amended; new claim 11 added; remaining claims unchanged (3 pages)]

1. A polymeric micelle drug composition capable of solubilizing a hydrophobic drug, which comprises: a micelle of a block copolymer having a hydrophilic compone and a hydrophobic component, and a hydrophobic drug physically incorporated into the micelle; wherein the hydrophobic component is a biodegradable polymer selected from the group consisting of polylactide, polyglycolide, poly(lactide glycolide), polycaprolactone, and a mixture thereof; and the hydrophilic component is poly(alkylene oxide).

2. The polymeric micelle drug composition of claim 1, wherein the block copolymer is a polymer of formula (I) or (II):

$$R_1\text{-}(\text{-}OCH_2CH_2\text{-})_m\text{-}X \qquad (I)$$

$$X\text{-}(\text{-}OCH_2CH_2\text{-})_m\text{-}X \qquad (II)$$

wherein

R_1 is hydrogen or C_{1-20} alkyl;

m is an integer ranging from 2 to 3,000; and

X is a polymeric segment having a molecular weight ranging from 100 to 100,000, which is selected from the group consisting of polylactide(PLA), polyglycolide(PGA), poly(lactide glycolide)(PLGA), polycaprolactone(PCL) and derivatives thereof.

8. The polymeric mic lle drug composition of claim 1, wh rein th hydrophobic drug is paclitax l and th hydrophobic component is polylactide or polycaprolactone.

9. A process for preparing a polymeric micelle drug composition capable of solubilizing a hydrophobic drug, which comprises the steps of:

preparing a micelle solution of a block copolymer having a hydrophobic component and a hydrophilic component, the hydrophobic component being a biodegradable hydrophobic polymer selected from the group consisting of polylactide, polyglycolide, poly(lactide glycolide), polycaprolactone and a mixture thereof, and the hydrophilic component being poly(alkylene oxide);

mixing the hydrophobic drug with the block copolymer solution;

subjecting the resulting mixture to stirring, heating, ultrasonic treatment, solvent evaporation or dialysis to physically incorporate the hydrophobic drug into the block copolymer micelle; and

filtering the mixture to recover the polymeric micelle-hydrophobic drug composition.

11. The process of claim 10, wherein the hydrophobic drug is paclitaxel, doxorubicin or cyclosporin.

FIG. 1

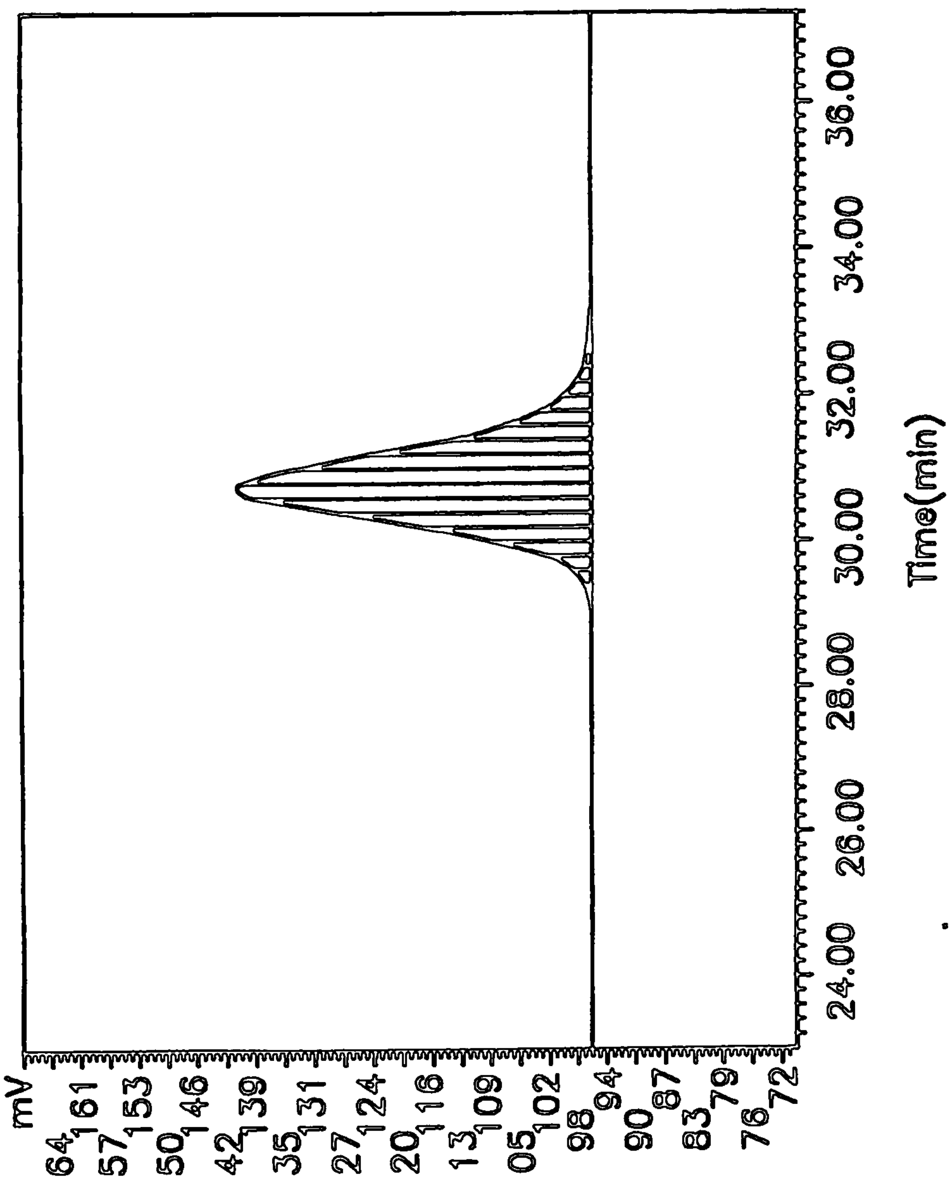
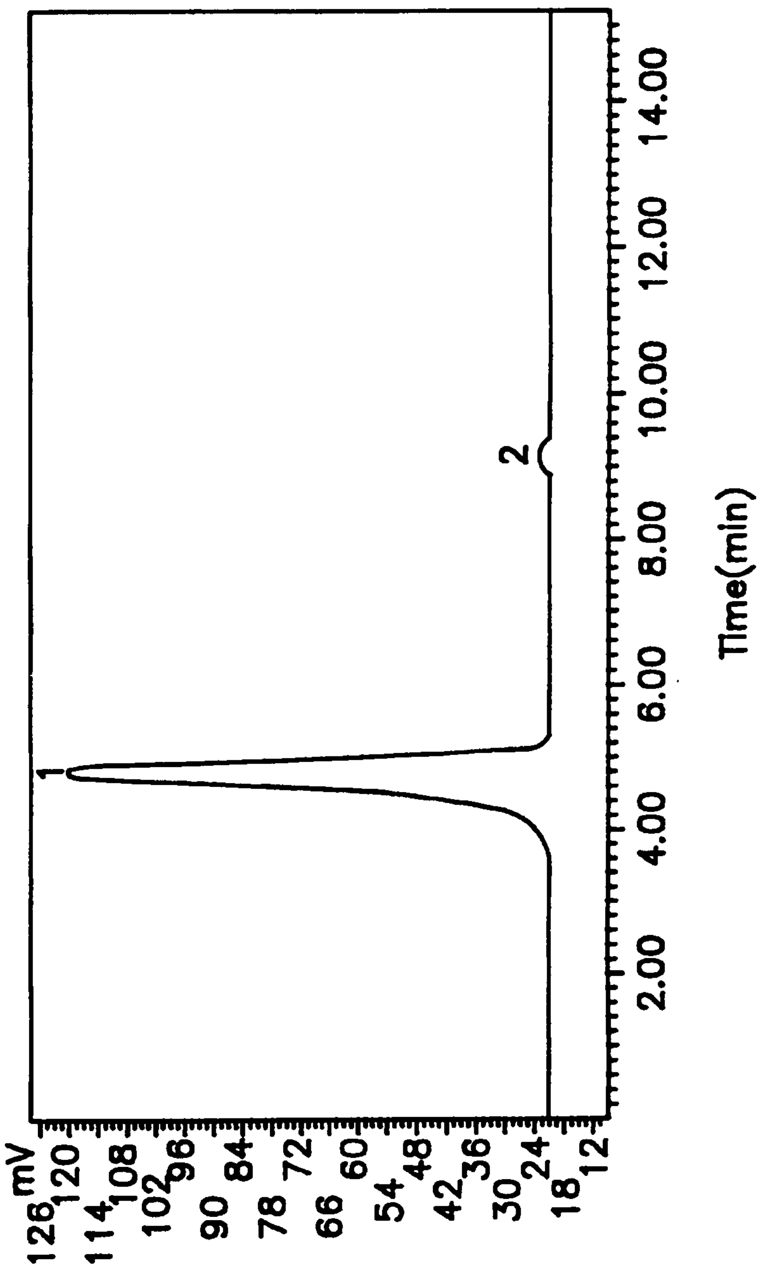

mV
164 161 157 153 150 146 142 139 135 131 127 124 120 116 113 109 105 102 98 94 90 87 83 79 76 72
24.00 26.00 28.00 30.00 32.00 34.00 36.00
Time(min)

FIG. 2


mV
126
120
114
108
102
96
90
84
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72
66
60
54
48
42
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30
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18
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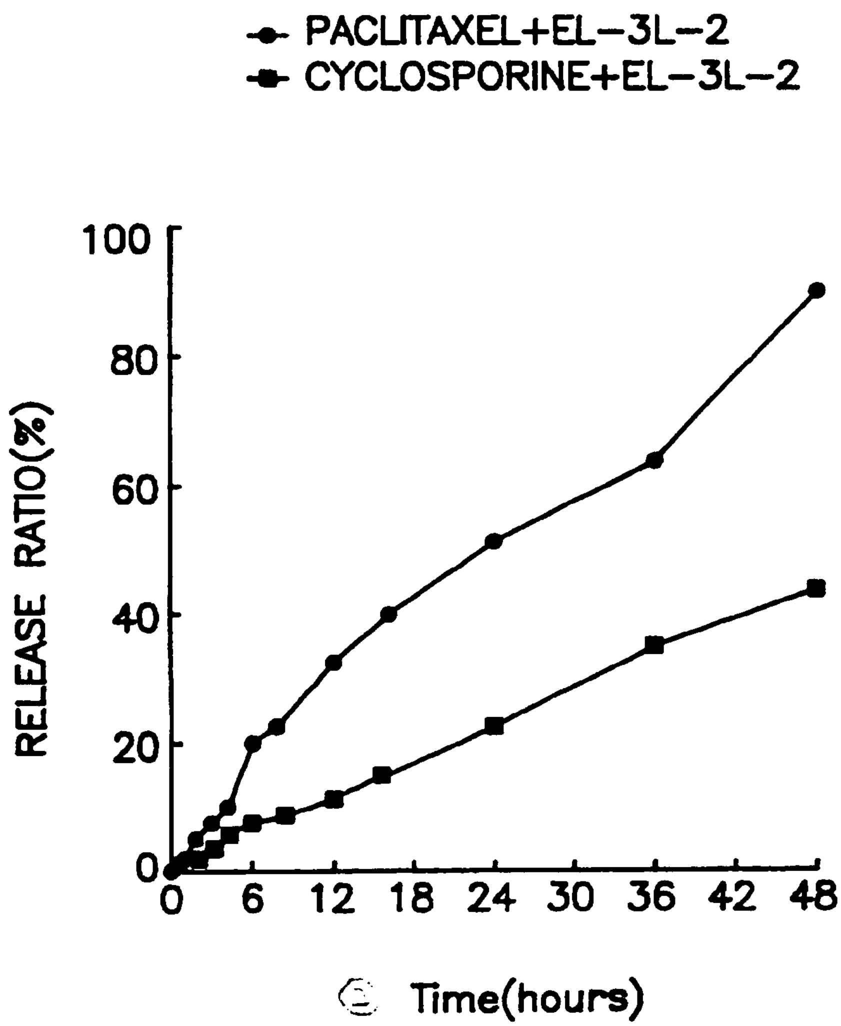
1
2
2.00
4.00
6.00
8.00
10.00
12.00
14.00
Time(min)

FIG. 3


PACLITAXEL+EL-3L-2
CYCLOSPORINE+EL-3L-2
RELEASE RATIO(%)
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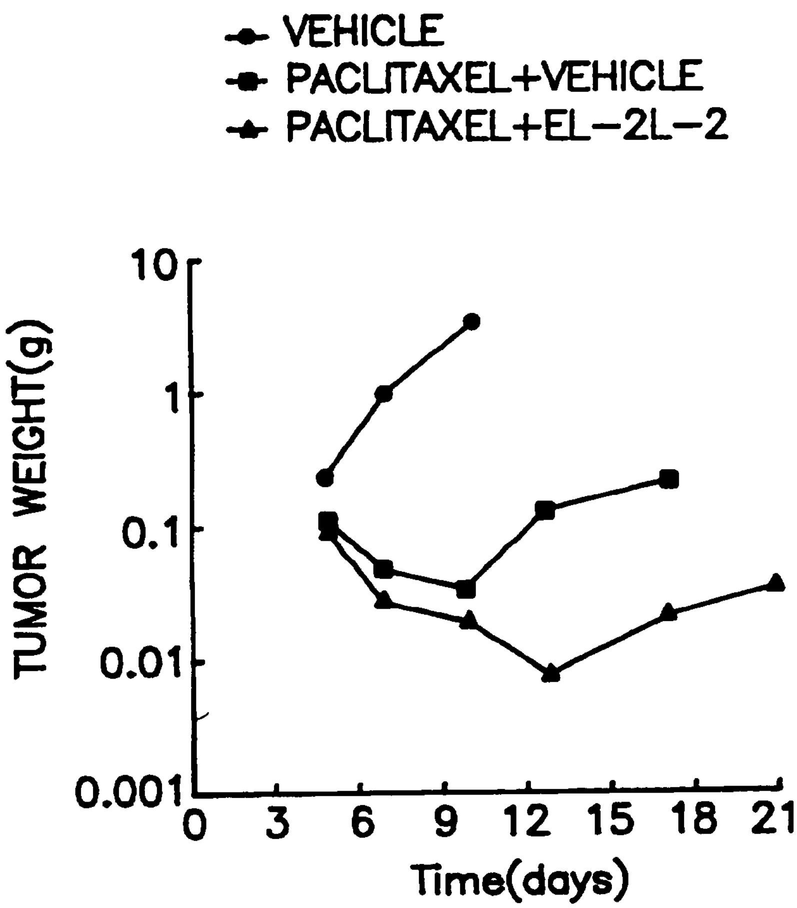
80
60
40
20
0
0
6
12
18
24
30
36
42
48
Time(hours)

FIG. 4



VEHICLE
PACLITAXEL+VEHICLE
PACLITAXEL+EL-2L-2
TUMOR WEIGHT(g)
10
1
0.1
0.01
0.001
0
3
6
9
12
15
18
21
Time(days)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR 96/00163

A. CLASSIFICATION OF SUBJECT MATTER

IPC^6: A 61 K 47/34, 9/51, 9/127

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC^6: A 61 K 47/00, 9/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Questel (FI CAS, FI WPIL)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category°	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95/03 357 A1 (MASSACHUSETTS INSTITUTE OF TECHNOLOGY) 02 February 1995 (02.02.95), abstract; claims 1-3, 11-13,15-17,19,20; page 31, lines 6-32; examples 2,4,5; page 35, lines 19-30.	1-3,5,9,10
X	US 5 384 333 A (DAVIS P.A. et al.) 24 January 1995 (24.01.95), abstract; claims 1-10; column 3, line 43 - column 5, line 48.	1-7
X,Y	EP 0 166 596 A2 (IMPERIAL CHEMICAL INDUSTRIES PLC) 02 January 1986 (02.01.86), claims 1-5,7,9,10; page 6, lines 1-32; page 9, line 13 - page 10, line 9.	1-5,9,10
Y	EP 0 092 918 A2 (IMPERIAL CHEMICAL INDUSTRIES PLC) 02 November 1983 (02.11.83), claims 1,4,5,7; page 5, last paragraph to page 6, line 16; page 7, lines 18-24.	1-5,9,10
X	EP 0 552 802 A2 (EASTMAN KODAK COMPANY) 28 July 1993 (28.07.93), claims 1-5,9; page 2, lines 29-57; page 3, lines 1-29; page 4, lines 16-21.	1-5,9

☐ Further documents are listed in the continuation of Box C.   ☒ See patent family annex.

° Special categories of cited documents:

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Date of the actual completion of the international search	Date of mailing of the international search report
12 November 1996 (12.11.96)	22 November 1996 (22.11.96)

Name and mailing address of the ISA/ AT	Authorized officer
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